Biological Polymers: Confined, Bent, and Driven

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1. Introduction

Cells exert and resist mechanical forces. This ability allows them to perform many essential tasks. Some cells can crawl across surfaces and through small pores, pulling themselves forward while pushing against their environment. Some cells swim by beating long appendages which push the surrounding fluid. Most cells proliferate by dividing into two daughter cells, which is accomplished by pinching the cell membrane at (most often) the mother cell's equator. Many cells maintain their internal components organized by a combination of internal pushing and pulling forces. Cell growth, division, and changes in shape allow fast-growing embryos to properly develop into organisms with a well-defined anatomy. But how can cells exert and withstand such forces?

In order to accomplish force-related tasks, cells rely on a variety of *biological polymers*. The kind of biological polymer used depends on cell type. Most plant, yeast, and bacterial cells maintain relatively constant, rod-like shapes. These cells possess an outer *cell wall* composed of rigid polymers, which provide robust mechanical stability. In contrast, many animal cells are soft and deformable. This allows them to move and change shape. Rather than possessing a static, rigid cell wall, animal cells rely on the *cytoskeleton* to provide resistance to external forces. However, at the same time the cytoskeleton itself also actively generates forces. These dynamic, adaptable proteins greatly contribute to the structural complexity of cells. Understanding the physical properties of biological polymers like cytoskeletal filaments is thus crucial in order to resolve the role of forces in cell mechanics.

In order to resolve how biological polymers regulate cell shape and mechanics, researchers in recent years have turned to quantitative experiments on purified biological polymers in a simplified, cell-free environment (Bausch and Kroy, 2006; Fletcher and Geissler, 2009).

The advantage of such biomimetic systems is that their molecular and structural complexity can be precisely controlled. The reduced complexity compared to living cells makes it easier to develop physical theories that predict the macroscopic physical properties in terms of the molecular properties of the components.

Yet our current knowledge of the mechanical properties of cytoskeletal polymers does not suffice in providing a complete mechanical description of how cells resist and exert forces. So far, much research has focused on the mechanical properties of single polymers, entangled networks and liquid crystals, crosslinked networks, and motor-driven systems. Yet there are many aspects of these polymer systems that remain poorly understood. Experiments so far have addressed the properties of macroscopic networks of biological polymers, yet we know little about how these networks are affected when spatially restricted to cellular dimensions. Furthermore, the formation of constricting rings is essential for cell division, yet how biological polymers form rings remains a mystery. Finally, the ability of biological polymers to exert forces is well understood at the microscopic level of single molecules, but how these forces can be integrated over long distances to give rise to larger forces remains poorly understood.

In this thesis, we aim to better understand the properties of the organization and force-generating capabilities of biological polymers. In order to achieve this goal, we investigate how biological polymers organize in cell-size confinement, how they can be bent into rings by crosslinks, and how they can actively exert forces over long distances. In all these cases, we present experimental results which current physical models fail to predict. A better theoretical understanding of our findings should lead to a more quantitative understanding of how forces influence cell behavior.

In this chapter, we present the state of the art in our understanding of biological polymers. First, we shall look at examples of biological polymers in living systems. Next, we shall investigate the properties of individual polymers, networks of polymers, crosslinked polymers, and actively driven polymers. Finally, the chapter will end with an outline of this thesis.

1.1 The cytoskeleton

The cytoskeleton is a network of biological polymers which provides cells with mechanical strength and the ability to generate active forces. Cytoskeletal polymers associate with a variety of accessory proteins to form different structures which execute distinct tasks. Despite the large number of possible cytoskeletal structures, the cytoskeleton primarily comprises only three types of polymers. In this section, we will introduce these cytoskeletal polymers and highlight some of the structures which they form. In later sections, we will investigate the properties of these polymers and some of their accessory proteins in more detail.

Microtubules are stiff polymers which help organize the interior of the cell. Microtubules act as tracks for accessory proteins called *molecular motors*, which move along microtubules to transport intracellular cargo. In interphase animal cells, microtubules usually radiate from the nucleus and extend toward the cell membrane, enabling transport between different parts of the cell (Barlan et al., 2013; Vale, 2003). For example, many amphibians and fish possess cells called *melanophores* which allow them to change color (Tuma and Gelfand, 1999). This is accomplished by molecular motors, which spatially rearrange vesicles containing the pigment melanin across microtubules. Before cell division, microtubules reorganize to form the *mitotic spindle*, an assembly of microtubules, molecular motors, and other accessory proteins which reliably separates chromosomes to the two daughter cells (Walczak and Heald, 2008). Fission yeast cells similarly use microtubules and molecular motors to separate chromosomes and transport cargo (Hagan, 1998). However, unlike in animal cells, interphase microtubules only extend to the two ends of the rod-shaped yeast cell. This allows molecular motors to deliver growth factors specifically to these two ends, maintaining yeast cells' rod-like shape (Chang and Martin, 2009). Some eukaryotic cells swim by beating *flagella* or *cilia*. These are long appendages which comprise an ordered arrangement of microtubules which slide past one another, causing the entire appendage to lash back and forth (Brokaw, 1994). In many plant cells, microtubules form an ordered *cortex*, or thin layer underneath the cell membrane. These microtubules help guide the

ordered production of the cell wall, which is essential in maintaining plant cells' elongated shape (Bringmann et al., 2012).

Actin filaments are more flexible polymers which can form either fine meshworks, branched networks, or stiff bundles. The most well-known example of actin filaments in organisms is found in muscle cells (Rayment et al., 1993). A well-organized array of actin filaments and molecular motors can exert contractile forces, allowing organisms to move, change shape, and drive essential functions like heartbeats and breathing. But non-muscle cells also possess an actin cytoskeleton, which can be used to exert forces, both internally and externally. In many animal cells, actin filaments form a thin *cortex* meshwork. This actin cortex allows molecular motors to exert forces which control cell shape (Salbreux et al., 2012). Furthermore, the actin cortex allows tissues of epithelial cells to exert forces on each other, maintaining tissue integrity (Cavey and Lecuit, 2009) and determining tissue shape (Rauzi and Lenne, 2011). The actin cortex also assists yeast and animal cells during *endocytosis*, the process whereby cells internalize foreign objects or fluids (Engqvist-Goldstein and Drubin, 2003). One example is *phagocytosis*, where immune cells engulf and destroy invasive pathogens like bacteria (May and Machesky, 2001). During eukaryotic cell division, cortical actin filaments and molecular motors organize into a *contractile ring*, which constricts to pinch off the mother cell into two daughter cells (Guertin et al., 2002). Apart from a thin cortex, some large cells such as oocytes additionally have a three-dimensional, cytoplasmic network of actin filaments (Field and Lénárt, 2011) which can be used for transporting chromosomes (Lénárt et al., 2005). Crawling cells like fish keratocytes, amoebas, and metastatic cancer cells can move across surfaces using a combination of actin-based structures (Abercrombie, 1980; Ananthakrishnan and Ehrlicher, 2007; Rafelski and Theriot, 2004). At the front of crawling cells, a thin, two-dimensional array of actin filaments called the *lamellipodium* pushes the cell membrane forward. Actin bundles called *filopodia* often accompany the lamellipodium, which can sense environmental cues that guide the direction of cell motion (Davenport et al., 1993). At the back of crawling cells, a network of actin filaments and molecular motors exerts retraction forces which allows the cell body to move forward. Inside the ear, inner hair cells project *stereocilia*, bundles of actin which participate in the transduction

of sound waves to neuronal impulses (Manor and Kachar, 2008). Interestingly, most plant cells lack an actin cortex. Rather, actin bundles usually radiate from the nucleus towards the cell membrane (Hussey et al., 2013) and assist in properly positioning the nucleus (Starr and Han, 2003).

Intermediate filaments provide animal cells with mechanical strength. They are encoded by 70 genes in the human genome, which are divided into six groups based on sequence homology (Szeverenyi et al., 2008). Different intermediate filaments are expressed in different cell types (Helfand et al., 2003; Herrmann et al., 2007). For example, epithelial cells such as skin cells resist deformation by a network of *keratin filaments* (Omary et al., 2009). Eukaryotic cells use *lamin filaments* to not only provide the nucleus with with mechanical strength, but also to regulate nuclear events such as chromosome replication and cell death (Gruenbaum et al., 2000). Fiber cells of the vertebrate eye lens contain *beaded filaments* which not only provide the lens with mechanical strength, but also maintain its transparency (Song et al., 2009).

Septin filaments have only recently begun to gain recognition as a fourth component of the cytoskeleton (Mostowy and Cossart, 2012). In budding yeast cells, septins form rings at the bud neck which separate the membranes of the mother and daughter cells (Byers, 1976; Hartwell, 1971). In animal cells, septins are core components of the contractile ring that are essential for proper cell division (Glotzer, 2005). However, their role in cytokinesis remains poorly understood.

1.2 Single filaments

So far we have seen many examples of cytoskeletal polymers and the structures they form inside living cells. In this section, we will introduce the properties of polymer filaments. We will see that cytoskeletal polymers possess unique properties that distinguish them from other biological polymers as well as synthetic polymers.

Macromolecular polymers. Many materials nowadays are made of plastics, which are *polymers* (Rubinstein and Colby, 2003), such as polyethylene, polystyrene, and polyvinyl chloride (PVC). The word "poly" in these names refers to a fundamental property of polymers: they comprise many copies of the same building block. These subunits assemble into long, linear chains. Polymer subunits within a chain are held together by strong covalent bonds. Individual polymer chains are thus large molecules, and often referred to as *macromolecules*.

Cells also produce macromolecular polymers, using sugars, nucleic acids and amino acids as building blocks. Plant cells produce cellulose fibers, built from linked glucose chains, which form a strong cell wall (Somerville, 2006). Bacterial colonies secrete extracellular polysaccharide chains which maintain cohesion and contribute to the formation of biofilms such as dental plaque (Costerton et al., 1999). Cells store their genetic information in the form of deoxyribonucleic acid (DNA), which are chains built from four different types of interchangeable nucleic acids (Alberts, 2008). Cells express DNA to produce proteins, which are macromolecules composed of one or more polypeptide chains, which themselves are long chains of up to twenty interchangeable amino acids (Alberts, 2008).

Supramolecular polymers. Many types of biological polymers are built up from many protein subunits. Unlike man-made plastics, these *supramolecular polymers* are formed via weak interactions such as electrostatic interactions, hydrophobic interactions, and hydrogen-bonding. The specificity of these interactions results in highly ordered structures. The non-covalent nature of these interactions lead to dynamic, regulatable structures. Collagen proteins assemble into thick fibrils that form the connective tissues of animals (Prockop and Kivirikko, 1995). Fibrinogen proteins also assemble into thick fibrils that form clots at wounds which stop bleeding (Weisel, 2008). Both of these fibril types are stabilized by covalent crosslinks, forming stable structures that often span far beyond cellular length scales.

Cytoskeletal polymers are also supramolecular polymers, but unlike collagen and fibrin polymers they are not covalently crosslinked. Because the subunits are held together by many weak, non-covalent interactions, cytoskeletal polymers can assemble and disassemble in response to biochemical signals. They can form dynamic and

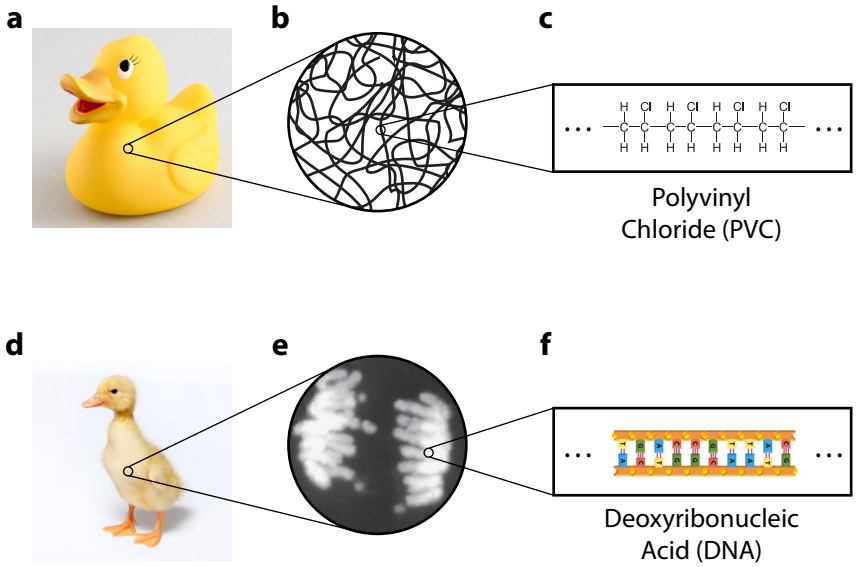

Figure 1. Common examples of polymers. **a**. A duck made of synthetic polymers. **b**. Polymers are long, linear chains. **c**. The structure of polyvinyl chloride (PVC), which comprises a long chain of vinyl chloride molecules. **d**. A duck made of biological polymers (among other materials). **e**. The nucleus of a dividing cell. **f**. The structure of an example strand of deoxyribonucleic acid (DNA), one of the most prevalent biological polymers. (Panels a, d © Wikimedia Commons. Panels e, f © Garland Science.)

adaptable structures that can allow cells to quickly respond to changing environments.

Polymer and subunit structure. Actin filaments comprise globular actin protein monomers. Actin monomers comprise two domains separated by a cleft, which binds a divalent cation as well as either adenosine triphosphate (ATP) or adenosine diphosphate (ADP). Monomers assemble head-to-tail to form linear polymer filaments. The ligand-binding cleft is directed toward the so-called "minus end" or "pointed end" of the filament. The opposite side is directed toward the "plus end" or "barbed end". Apart from assembling head-to-tail, actin

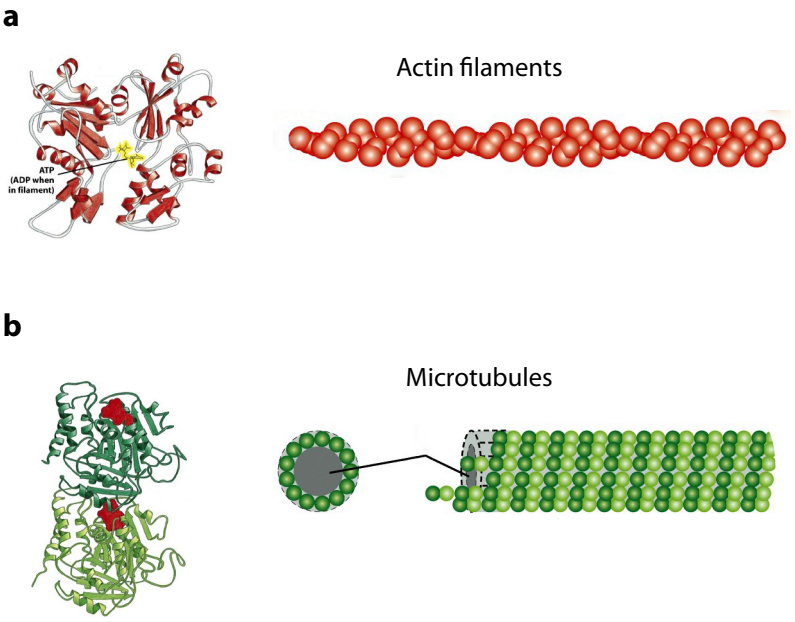

Figure 2. Actin and microtubules. **a**. Molecular structure of actin monomers, which assemble into actin filaments. **b**. Molecular structure of α- and β-tubulin, which form dimers that assemble into microtubules. (Panels a, b © Garland Science.)

monomers associate via side-by-side contacts, forming a two-stranded helical structure with a 37-nm pitch.

Microtubules comprise α- and β-tubulin proteins, which form stable heterodimers. β-tubulin proteins bind guanine triphosphate (GTP) or guanine diphosphate (GDP). Although α-tubulin proteins bind guanine triphosphate (GTP), this binding site is buried at the dimer interface. Dimers of α-β-tubulin assemble head-to-tail to form linear protofilaments, with α-tubulin at the "plus end" and β-tubulin at the "minus end". Thirteen protofilaments associate side-by-side to form a hollow, cylindrical microtubule. This stable, tubular structure makes microtubules stiffer than actin filaments by a factor of approximately 300 (Gittes et al., 1993).

The notion of head-to-tail assembly indicates a special property of actin filaments and microtubules. S*tructural polarity* refers to the fact that the two ends of the filament can be distinguished from each other. Other examples of polymers with structural polarity are DNA (5' and 3' ends) and protein polypeptide chains (N- and C-termini). This is in strong contrast to many other synthetic and biological polymers, which exhibit *structural symmetry*. In structurally symmetric filaments, both ends of the filament are identical and indistinguishable. Intermediate filaments and septin filaments are structurally symmetric.

The amino acid sequences of actin and tubulin proteins are surprisingly well conserved across many eukaryotic species (Mitchison, 1995; Sheterline and Sparrow, 1994). Intermediate filaments and septin filaments also maintain a large degree of evolutionary conservation, although species-specific variation is greater than with actin and tubulin. Even in prokaryotes, various actin, tubulin, and intermediate-filament homologues been identified which can also assemble into polymers to perform a variety of tasks (Shih and Rothfield, 2006).

Polymerization and enzymatic activity. The process by which monomer subunits join a polymer is called *polymerization*. For supramolecular polymers such as actin, this process is characterized by the rate of monomer addition, k_{on}, as well as the rate of monomer dissociation, k_{off}. In equilibrium, these rates are identical. For structurally polar filaments, the on/off rates vary for the two different ends. The end with the higher rate is conventionally called the "plus end", while "minus end" refers to the end with the slower rate. Structurally symmetric filaments have identical on/off rates at both ends.

Actin and microtubules, apart from possessing structural polarity, also possess enzymatic activity. These proteins bind the nucleoside triphosphates ATP or GTP. Shortly after subunits join polymers, hydrolysis occurs. Continuous addition of fresh ATP- or GTP-bound monomers therefore results in a so-called *ATP cap* or *GTP cap* at the plus end, while the rest of the filament contains ADP- or GDP-bound monomers. The presence of such a cap allows for faster polymerization kinetics at the plus end, which can lead to dynamic, non-equilibrium processes such as treadmilling (Pollard and Borisy, 2003) and dynamic instability (Gardner et al., 2011). Specialized tip-tracking molecules can selectively bind at the plus end of microtubules, though it remains

unclear whether they bind specifically to the GTP cap or to other nucleotide-bound states (Bowne-Anderson et al., 2013; Maurer et al., 2012; Seetapun et al., 2012). These dynamic properties allow cytoskeletal filaments to exert polymerization and depolymerization forces, which we will discuss later in this chapter.

Although intermediate filaments lack structural polarity and enzymatic activity, evidence suggests that intermediate filaments exhibit fast polymerization and depolymerization kinetics (Helfand et al., 2003). Septin filaments lack structural polarity but exhibit enzymatic activity (Weirich et al., 2008). Septin subunits bind and slowly hydrolyze GTP, and septin subunits can form filaments via the GTP-binding domain (or also at an interface containing N- and C-termini). However, the role of enzymatic activity in regulating filament formation remains poorly understood.

Worm-like chain model. So far we have seen how molecular structure can determine many of the special properties of cytoskeletal filaments. Yet physical theories accounting for the mechanical properties of polymer filaments often ignore fine structural detail. The *worm-like chain model* is the most common theoretical model used to describe the mechanics of cytoskeletal polymers. This model was originally developed by Kratky and Porod (Kratky and Porod, 2010). It approximates polymers by a smooth linear contour which resists bending with a bending modulus κ. In the absence of thermal fluctuations, polymers assume a linear shape. But at finite temperatures, random forces from thermal fluctuations can cause the polymer to bend. These thermal bending undulations have been observed experimentally for actin filaments by fluorescence microscopy (Gittes et al., 1993; Isambert et al., 1995; Ott et al., 1993) and have been used to measure κ. This was achieved by measuring a length scale l_p called the *persistence length*, which is defined as the decay length of angular correlations along the polymer contour. Roughly speaking, the persistence length is the distance over which the polymer contour appears straight. In equilibrium, the persistence length is related to the bending modulus by the relation $\kappa = kT\, l_p$, where k is Boltzmann's constant and T is temperature.

Using the persistence length l_p and the contour length L, we can distinguish between three classes of polymers. If the polymer backbone offers little resistance to bending ($l_p \ll L$), thermal fluctuations

dominate, bending the polymer so strongly that it crumples to a highly bent conformation well described by a fractal contour (de Gennes, 1979). Such polymers are called *flexible polymers*, and are suitable for describing many synthetic macromolecular polymers. In the opposite scenario ($l_p \gg L$), *stiff polymers* strongly resist thermal fluctuations and can be modeled as rigid rods (Landau et al., 1986). A third intermediate regime occurs when $l_p \sim L$. In this regime, thermal fluctuations cannot be neglected, though the polymer retains a well-defined, mostly straight shape with long, wavelike undulations. Polymers in this intermediate regime are called *semiflexible polymers*. Many biological polymers are semiflexible. Double-stranded DNA has a persistence length of 50 nm (Hagerman, 1988). Cytoskeletal filaments have a much larger persistence length ranging from 0.5-1 μm for intermediate filaments, to 8 μm for actin filaments and 5 mm for microtubules (Kasza et al., 2007).

Response to pulling forces. So far we have seen that the mechanical properties of semiflexible filaments can be characterized by the persistence length. This quantity describes how filaments respond to thermal forces. But how do semiflexible polymers respond to external pulling forces? Given an infinitely strong force, we should expect the polymer to assume a straight shape: such a taut filament would not bend due to thermal forces. Theoretical models have accounted for the reduction of thermally-induced bends due to external pulling forces (MacKintosh et al., 1995). The amplitude of thermally induced bends in the polymer depends on wavelength, given by the wave vector $q = n\,\pi / L$, where $n = 1, 2, 3\ldots$ If the polymer experiences tension due to an external pulling force f, the amplitude u_q of bending mode q is given by

$$\left\langle \left| u_q \right|^2 \right\rangle = \frac{2kT}{L\left(\kappa q^4 + f q^2\right)}$$

Long-wavelength bends (lower q) have the largest bending amplitudes in equilibrium. Short-wavelength bends (higher q) decay quickly, as q^{-4}. This formula also shows that applying a pulling force f to the polymer reduces bending amplitudes u_q. This reduction in thermal modes results in an effective restoring force:

$$f \sim \frac{l_p \kappa}{L^4} x$$

where x denotes the displacement of the end-to-end-distance vector of the polymer contour from its equilibrium position. The effective spring constant is thus $l_p\, \kappa\, L^{-4}$. Because semiflexible polymers bend in response to thermal forces, their response to a pulling forces is entropic in origin. This effect is often called the *entropic spring*. The force-extension relation was experimentally verified with DNA in optical tweezers (Bustamante et al., 1994), as well as for actin and microtubules (van Mameren et al., 2009).

So far we have considered how pulling forces decrease the bending amplitudes of polymer fluctuations. Note that this expression only takes into account the bending response of the polymer. Interestingly, this result is valid even though we have completely neglected the enthalpic response of the polymer chain itself to stretch deformations. However, the above equations are only valid under the assumption of small forces and linear responses. For strong pulling forces the thermal undulations are pulled out, and this assumption breaks down. In this case, we must account for stretch deformations of the polymer chain, which slightly elongates their contour. This has been accomplished by introducing a stretch modulus μ in the worm-like chain model (Odijk, 1995; Storm et al., 2005). The result is the emergence of two different force-response regimes with distinct spring constants. For low deformations, the effective spring constant is dominated by the bending modulus κ according to the entropic spring. For high deformations, the effective spring constant is dominated by the stretching modulus μ, corresponding to the enthalpic stretch of the polymer contour.

Response to pushing forces. We have investigated how individual semiflexible polymers respond to pulling forces. But how do they respond to pushing forces? Given small forces and linear responses, the stretch modulus μ determines the compressive deformation of elastic rods. Long, semiflexible rods can readily undergo a *buckling* instability when pushing forces exceed the critical Euler force f_c (Landau et al., 1986):

$$f_c \sim \kappa\, L^{-2}$$

This force is the maximal protrusive force that a rod can exert. Increased external pushing forces do not result in further compressive deformations. Rather, the rod bends and gives way, ultimately resulting in its collapse.

Microtubules are stiff polymers and can withstand rather high compressive forces. Actin filaments buckle at forces 300 times smaller, owing to their reduced bending stiffness (Gittes et al., 1993). This results in an *asymmetry in mechanical response*: single actin filaments can withstand and propagate pulling forces but not pushing forces. Yet despite this asymmetry, we will later see that cells can overcome this limitation and use actin filaments to exert substantial pushing forces (see "Force generation", below)

1.3 Filament networks

In cells, biological polymers are generally present at high density. So far we have encountered mechanical descriptions of single filaments. In this section, we will describe the collective properties of materials composed of many filaments. In order to accomplish this, we shall first investigate the phase behavior of suspensions of rigid rods. These model systems have been theoretically well characterized, and form the basis of understanding the properties of filament networks.

Rigid rods: dilute, entangled, and nematic regime. Consider a molecular rod of length L and thickness d diffusing freely in solution. As it translates and rotates, it sweeps out a volume $\sim L^3$. Thus, for a suspension of rods with concentration $c \ll L^{-3}$, neighboring rods are spaced far enough apart that they do not significantly interfere with each other's motions. In this *dilute* regime, the rotational diffusion constant $D_{\circlearrowleft}$ scales with rod length (with a prefactor that depends on temperature and viscosity) (Riseman and Kirkwood, 1950):

$$D_{\circlearrowleft,\text{dilute}} \sim L^{-3} \ln(L/d)$$

If the rod concentration c increases beyond L^{-3}, rods interact via steric repulsion: two rods cannot overlap in space, and therefore repel each other upon contact. In this *entangled* regime, the diffusion of a rod is constrained by its neighbors. Early theory by Doi and Edwards modeled the effect of entanglements for concentrated suspensions of rods by

proposing the tube model (Doi and Edwards, 1978). In this model, a rod of interest cannot diffuse freely in a volume $\sim L^3$, but is rather confined to an elongated virtual tube formed by the presence of neighboring rods. This results in a drastically reduced rotational diffusion constant for the entangled regime:

$$D_{\circlearrowleft,\text{entangled}} \sim L^{-6} D_{\circlearrowleft,\text{dilute}}$$

If the rod concentration c increases further towards $d^{-1}L^{-2}$ (where $d\ L^2$ is the volume of a single rod), the packing of rods cannot be neglected. Early theory by Onsager in this dense limit found that rods self-align to decrease their mutual excluded volume (Onsager, 1945). At concentrations below a critical concentration c^*, rod orientations are isotropically distributed to maximize rotational entropy. At concentrations above c^*, rod orientations are distributed about a preferred direction. Although this orientational alignment decreases rotational entropy, it is compensated by an increase in translational entropy due to a decrease in the mutual excluded volume. In the limit of infinitely long rods, the critical concentration c^* depends only on the inverse of the rod aspect ratio D / L. This phase of matter is called a *nematic* phase. The orientational anisotropy of rods in the nematic phase results in an anisotropy in optical properties, giving the appearance of a crystal. Hence, materials in the nematic phase form one of many possible *liquid-crystalline* phases.

Polymer networks. So far, we have considered theories which treat suspensions of diffusing rigid rods of uniform length. These theories have successfully predicted the phase behavior of suspensions of rod-shaped colloidal objects, including the rodlike viruses tobacco-mosaic virus (Graf and Löwen, 1999) and bacteriophage fd (Dogic and Fraden, 2006). However, polymers are usually neither rigid nor uniform in length. Theories have been developed to predict the changes in phase behavior arising from these deviations. In particular, the critical concentration c^* needed to form a nematic phase increases for both flexible filaments (Khokhlov, 1982) as well as filaments with nonuniform lengths (Odijk, 1986; Odijk and Lekkerkerker, 1985).

Yet despite these quantitative differences, the overall phase behavior predicted by theories on uniform, rigid rods provides a good description of the phase behavior of networks of rigid polymers such as actin and microtubules. For entangled actin filament networks with

actin concentrations between 0.1 to 2 mg mL^{-1}, the tube model was confirmed experimentally (Käs et al., 1996): labeled filaments were observed to fluctuate within a virtual, confining tube formed by the unlabeled surrounding filaments. Above concentrations of ~2 mg mL^{-1}, networks of actin filaments can form nematic phases (Käs et al., 1996; Suzuki et al., 1991). Shortening actin filaments by adding the capping protein gelsolin increases c^*, consistent with Onsager's theory (Suzuki et al., 1991).

Mechanical properties of entangled polymer networks. Entangled networks of polymer filaments form *viscoelastic* gels, which exhibit behavior characteristic of both solids and fluids. Rheology experiments quantify the mechanical properties of such viscoelastic materials. In these experiments, gels are grown between two large surfaces, which are moved relative to one another to apply shear stresses on the material. The response of a viscoelastic material to these shear stresses is given by two quantities (Meyers and Chawla, 2009): the storage modulus G', which measures elastic, or solid-like behavior; and the loss modulus G'', which measures viscous, or fluid-like behavior. These two quantities can be expressed as a single complex shear modulus $G = G' + i\,G''$.

For entangled actin networks, the primary determinant of the mechanical properties is the filament density. The storage modulus scales with concentration according to $G \sim \varphi^{7/5}$, where φ is the polymer volume fraction (Gisler and Weitz, 1999; Hinner et al., 1998). Theoretical scaling arguments, which consider two length scales, can account for this experimental result. The first length scale is the mesh size ξ, which is defined as the typical spacing between filaments. It scales as $\xi \sim \varphi^{-1/2}\, a$, where a is the thickness of a single filament (Schmidt et al., 1989). The second relevant length scale is the entanglement length l_e, which describes the typical length over which filament entanglements restrict thermal fluctuations. It scales as $l_e \sim (a^4\, l_p)^{1/5}\, \varphi^{-2/5}$, where l_p is the persistence length (Hinner et al., 1998; Isambert and Maggs, 1996). These two length scales determine the properties of networks of semiflexible filaments, with $G \sim kT / (\xi^2\, l_e)$. Substituting this expression with ξ and l_e yields the experimentally determined scaling relation $G \sim \varphi^{7/5}$. Interestingly, the two length scales, and thus the shear modulus

G, do not depend strongly on the stiffness of single filaments, given by l_p.

Entropic forces. So far we have explored how steric repulsion between filaments can account for different material phases, including entangled networks and nematic liquid crystals. In cells, the organization of actin filaments is further affected by steric interactions with other cytoplasmic components and with the cell membrane.

First, adding inert globular polymers can cause filaments to aggregate into bundles via the so-called *depletion attraction*. Globular polymers can be thought of as diffusing, impenetrable spheres. They interact with filaments mainly via steric repulsion. At a low concentration c of the globular polymer, filaments and polymers diffuse without significantly affecting each other. However, above a critical concentration c^* of polymer, filaments bundle together in order to maximize the free volume available to the globular polymers, thereby maximizing translational entropy (Lekkerkerker and Tuinier, 2011). Experiments have shown that actin filament networks indeed become bundled when sufficient amounts of inert polyethylene glycol (PEG) polymers are added (Hosek and Tang, 2004).

Second, filament organization can be strongly affected by the presence of external boundaries, leading to *confinement* effects. Initial theoretical work has addressed confinement effects by studying suspensions of rigid rods in the isotropic phase near an impenetrable planar surface. Rods were found to align along the surface, forming a so-called orientational wetting layer (van Roij et al., 2000). This effect only occurs for rods close to the surface, with an effective layer depth on the order of one rod length. In the case of semiflexible polymer networks, experiments investigated the effect of actin filament networks grown close to a rigid planar surface. The density of the network was found to decrease close to surfaces, forming a *depletion layer* whose thickness compares with the average filament length (Fisher and Kuo, 2009). Interestingly, the opposite effect was found when filaments were confined in three dimensions. Actin filaments grown in emulsion droplets were found to *accumulate* at the droplet surface, forming a cortex-like layer when droplets were smaller than the persistence length of actin filaments (Claessens et al., 2006b). Similarly, microtubules

grown in confining microchambers were found to coil and wrap around the chamber edges (Cosentino Lagomarsino et al., 2007).

Note that the depletion attraction and confinement effects induce only effective interactions between filaments. These interactions are mediated by the maximization of entropy of the entire system, similarly to the alignment of filaments in the nematic phase. For this reason, these indirect effects are referred to as *entropic forces*.

Entropic forces likely contribute to the organization of cytoskeletal structures inside cells. The environment inside most cells is crowded with soluble proteins which comprise 20–30% of the cell's volume (Ellis, 2001). For this reason, the depletion interaction has been suggested to contribute to actin filament bundling, amyloid fibril formation, and DNA looping (Marenduzzo et al., 2006). Similarly, confinement effects should also play a significant role in cytoskeletal organization. Cytoskeletal filaments have contours and persistence lengths that often compare with cellular dimensions, especially in thin compartments such as lamellipodia and filopodia. However, the extent to which these entropic forces determine intracellular organization remains poorly understood.

1.4 Crosslinks

So far, we have investigated how steric interactions affect the structure and mechanical properties of biological polymer networks. We neglected the effect of other important physical forces that can strongly affect phase behavior, including electrostatic attraction and repulsion, van der Waals interactions, and the free energy of hydration (Leckband and Israelachvili, 2001). In addition, cells possess *crosslink* proteins which specifically connect filaments to each other as well as to other cellular structures such as the plasma membrane. By tightly regulating the density and activity of these crosslink proteins, cells can create different cytoskeletal structures without significantly affecting the

physical properties of the filaments themselves. In this section, we will focus on *actin-binding proteins*, accessory proteins which crosslink actin filaments. We will describe their structural properties and consequences for the organization and mechanical properties of actin filament networks.

Crosslink structure. Most crosslink proteins contain at least two actin-binding domains. Each domain can independently bind a separate filament, thereby creating a mechanical link between filament pairs. Different types of actin-binding domains have been identified. The most common kind is the calponin-homology domain, which is found across a broad class of crosslink proteins, including spectrin, filamin, fimbrin, and α-actinin (Korenbaum, 2002). Fascin proteins bind actin filaments through β-trefoil domains (Jansen et al., 2011). Some crosslinks such as fascin and fimbrin can only bind pairs of parallel filaments, whereas other crosslinks such as α-actinin and filamin can bind actin filaments over a wide range of angles, forming isotropic rigid networks (Courson and Rock, 2010; Stossel et al., 2001).

Crosslink binding. Crosslinks bind with typical dissociation constants of 0.1–3 μM (Chen et al., 1999; Goldmann and Isenberg, 1993; Meyer and Aebi, 1990; Ono et al., 1997; Skau et al., 2011; Wachsstock et al., 1993; Yamakita et al., 1996). This corresponds to binding free energies of 32–42 kJ mol^{-1}, or 13–17 kT at room temperature. In equilibrium, crosslinks also unbind, with typical timescales of 10 s (Courson and Rock, 2010). Stresses acting on crosslinks usually accelerate crosslink unbinding (Evans and Ritchie, 1997). Such crosslinks are known as *slip bonds*. However, thecrosslink α-actinin 4 exhibits different stable conformations (Galkin et al., 2010a), which can expose additional actin binding domains buried inside the crosslink (Volkmer Ward et al., 2008) when subject to stress (Yao et al., 2011). Remarkably, these crosslinks therefore bind more tightly under tension, and are known as *catch bonds* (Thomas et al., 2008).

Crosslinked meshworks and bundles. Adding crosslinks to entangled actin networks can result in a variety of structures, including fine crosslinked meshworks, pure bundle networks, bundle cluster networks, and composite meshwork-bundle networks (Lieleg et al., 2010). However, predicting network structure given the local crosslink structure and binding mechanism remains elusive.

Mechanical properties of crosslinked networks. Introducing crosslinks in an actin filament network introduces a new length scale, called the crosslink distance l_c. This distance is the average separation between crosslinks. Similar to the entanglement l_e, this length scale determines the mechanical properties of crosslinked polymer networks. In particular, l_c determines whether a network deformation results in predominantly filament stretching or filament bending. WWhen a macroscopic shear strain results predominantly in filament stretching, the network experiences *affine* deformations (or *uniformly*). For affine thermal deformations, the shear modulus of the network depends on the concentration c_x of crosslinks (Gardel et al., 2004):

$$G_{\text{affine}} \sim c_x \kappa l_p l_c^{-3}$$

When filaments are very stiff, or when the network connectivity is low, filaments or bundles significantly bend when a macroscopic shear stress is applied, resulting in *nonaffine* deformations. In this case, the shear modulus of the network is insensitive to the concentration of crosslinks and instead depends strongly on the concentration of actin filaments c (Kroy and Frey, 1996):

$$G_{\text{nonaffine}} \sim \kappa \xi^4 \sim c^2$$

1.5 Force Generation

So far we have encountered passive physical forces that determine the organization and mechanical properties of biological polymers. But unlike many polymers, actin filaments and microtubules are out of equilibrium because of consumption of chemical energy in the form of the nucleotides ATP and GTP. We have already seen that these polymers exhibit enzymatic activity, which leads to polarized polymerization, treadmilling, and dynamic instability. These non-equilibrium properties allow actin filaments and microtubules to exert forces as they grow or shrink. Furthermore, motor proteins can slide filaments past one another, leading to generation of pushing and pulling forces. In this

section, we review the mechanisms whereby biological polymers can actively exert force.

Polymerization and depolymerization forces. Actin filaments and microtubules polymerize asymmetrically due to differences in the free energy of monomer binding between the plus-end and the minus-end. This free energy difference can be harnessed as filaments grow against a barrier to exert pushing forces (Hill, 1981; Theriot, 2000). Single microtubules are stiff and can exert forces of up to 3–4 pN as they polymerize (Dogterom and Yurke, 1997), though forces of up to ~50 pN should be possible (Dogterom et al., 2005). These forces are essential for maintaining the internal organization of the cell (Tolić-Nørrelykke, 2008), including the proper positioning of the kinetochore and chromosomes in the mitotic spindle in animal cells (Inoué and Salmon, 1995). Actin, too, exerts pushing forces. Despite the fact that single actin filaments alone are more flexible than microtubules and should buckle readily under compression at forces of ~0.1 pN (Landau et al., 1986), actin filaments have been measured to exert polymerization forces of up to 1 pN (Footer et al., 2007). Furthermore, actin-based structures can exert larger forces when organized by accessory proteins. Filopodia in the growth cones of migrating neurons, which contain actin bundles crosslinked by the protein fascin, can exert pushing forces of up to 3 pN (Cojoc et al., 2007). Such weak force generation may potentially serve to sense mechanical cues and preferentially grow along soft substrates (Betz et al., 2011). In the lamellipodium of crawling fish keratocytes, a dense array of short actin filaments branched by the Arp2/3 complex at the leading edge polymerize against the membrane and push it forward (Mogilner and Oster, 1996; 2003), exerting forces of about 100 pN (Roure et al., 2005). A similar mechanism is used by the bacterium *Listeria monocytogenes* (Cameron et al., 2001; Tilney and Portnoy, 1989). This pathogen uses the actin machinery of infected cells to propel itself with forces of 10–100 pN (McGrath et al., 2003; Wiesner, 2003). More dramatically, during the acrosomal process of the horseshoe crab *Limulus polyphemus*, a stiff bundle of actin filaments crosslinked by the protein scruin extends from sperm cells to break open the egg cell wall with a force of 2 nN (Shin et al., 2003; 2007).

Other biological polymers are similarly capable of exerting pushing forces. Sperm cells of the nematode *Ascaris suum* migrate using major

sperm protein (MSP) polymers, which elongate and pack in a similar fashion to the actin cytoskeleton in the lamellipodium (Miao et al., 2008; Roberts and Stewart, 2000). In *Escherichia coli* bacteria, the actin homologue ParM (Bork et al., 1992) polymerizes to push chromosomes apart to cell poles before division (Garner et al., 2007).

Apart from exerting pushing forces during polymerization, actin filaments and microtubules can exert pulling forces during depolymerization. In the case of microtubules, these forces are transmitted through tip-tracking proteins, which selectively bind the plus end of microtubules (Schuyler and Pellman, 2001). As microtubules shrink, tip-tracking proteins can remain bound to the retreating plus end (Lombillo et al., 1995). These forces are believed to underlie some of the pulling forces necessary for proper positioning of chromosomes during cell division (Dickinson et al., 2004; Hill, 1981; Joglekar et al., 2010; McIntosh et al., 2010). In the case of actin, filament depolymerization is essential during the constriction of the actomyosin ring during cytokinesis in the budding yeast *Saccharomyces cerevisiae* (Mendes Pinto et al., 2012).

Molecular motors. In addition to the ability of actin filaments and microtubules to exert polymerization and depolymerization forces, cells also possess specialized proteins called *molecular motors*. These proteins can exert forces by again coupling the free energy of ATP hydrolysis to mechanical work. This mechanical work can be harnessed for a wide variety of tasks, including DNA replication and expression, protein translocation, cell migration, chromosome separation, and cytokinesis (Bustamante et al., 2004).

Here we focus on the cytoskeletal motor proteins, which can exert forces while moving along cytoskeletal filaments. There are three classes of cytoskeletal motor proteins (Howard, 1997). *Myosin* motors bind actin filaments and most of the ca. 20 types of myosins move towards the plus-end. *Kinesin* and *dynein* motors bind microtubules and move towards the plus- and minus-end, respectively. Although there can be considerable variation among molecular motor types (Goodson et al., 1994; Thompson and Langford, 2002), cytoskeletal motor proteins share a few common design principles (Howard, 1997; Schliwa and Woehlke, 2003). They possess one or two head domains which bind filaments as well as ATP or ADP. Upon ATP hydrolysis, motor proteins undergo

conformational changes, manifested in a "power stroke" that results in step-wise motion of the motor along the filament. Step sizes typically vary between 8 and 30 nm, generating forces of up to ~10 pN (Burgess et al., 2003; Finer et al., 1994; Ishijima et al., 1998; La Cruz et al., 1999; Mehta et al., 1999; Visscher et al., 2000). Motor proteins also possess tail domains, which can bind to the tail domains of other motors to form oligomeric motor complexes (Bresnick, 1999), or to the cell cortex (Dujardin and Vallee, 2002), or to intracellular cargo (Hirokawa, 1998).

Many cells and organisms rely on molecular motors to exert forces that are stronger than by polymerization or depolymerization alone. Unicellular organisms such as the alga *Chlamydomonas reinhardtii* beat two long flagella composed of microtubules and dynein and kinesin motors (Bernstein and Rosenbaum, 1994), allowing the cell to propel itself with a force of 30 pN (McCord et al., 2005). Fish keratocytes glide on surfaces powered by myosin contraction, exerting traction forces of 45 nN (Harris et al., 1980; Oliver et al., 1995). Similar traction forces between kidney epithelial cells maintain tissue integrity and reach 100 nN (Maruthamuthu et al., 2011). Even higher forces can be achieved with dedicated muscle cells, which organize actin filaments and myosin motors in a sarcomeric structure dedicated to integrating the power strokes of many myosin motors (Gautel, 2011; Huxley and Niedergerke, 1954; Huxley and Hanson, 1954). Individual cardiac muscle cells have been measured to exert forces of 10 μN (Lin et al., 2000; Tarr et al., 1983; Yin et al., 2005).

Motor activity and spatial organization. Apart from exerting forces on their surroundings, cells use molecular motors to organize transient internal structures such as the mitotic spindle (Dumont and Mitchison, 2009; Tolić-Nørrelykke, 2008). Understanding how forces produced by single motors translate into cell-scale forces and cell-scale spatial organization remains an enormous challenge. Forces re-organize the cytoskeleton, but the spatial organization of the cytoskeleton in turn influences force generation. Addressing this feedback in living cells is hindered by their inherent complexity. Recent experiments with reconstituted cytoskeletal networks driven by molecular motors have started to address how spatial organization and force generation can affect each other.

Microtubules driven by kinesin and dynein motors exhibit fascinating structural patterns in solution, including vortices and asters (Nédélec et al., 1997). Similar asters have also been reported in the case of actin bundles driven by myosin motors (Backouche et al., 2006). In confined geometries, microtubule asters can be reliably centered by either pushing forces from microtubule polymerization (Holy et al., 1997) or pulling forces from dynein motors (Laan et al., 2012). In all these cases, self-organization arises from a feedback between force generation and the motion of stiff filaments. Meanwhile, single actin filaments are relatively flexible and readily buckle under compressive forces. This property is likely the reason why actin filament meshworks driven by myosin motors have not been reported to exhibit the same pattern formation as microtubules (Soares e Silva et al., 2011b). Buckling of actin filaments under compressive loads leads to an asymmetry in the response of actin networks to local internal forces generated by motors, biasing towards motor pulling forces and leading to contraction (Lenz et al., 2012b; Liverpool et al., 2009; Murrell and Gardel, 2012; Vogel et al., 2013).

Material properties of motor-driven systems. Apart from exerting forces and affecting spatial organization, molecular motors can also strongly affect the material properties of the polymer systems with which they interact. Myosin activity enhances fluctuations of crosslinked actin networks at frequencies below 10 Hz which violate the fluctuation-dissipation theorem (Mizuno et al., 2007) and cause strong non-Gaussian displacements of embedded probe particles (Stuhrmann et al., 2012). In suspensions of clusters of actin bundles, myosin motors can regulate cluster size (Köhler et al., 2011a) and lead to superdiffusive behavior (Köhler et al., 2011b). Stresses resulting from myosin activity stiffen crosslinked actin networks by a factor of 100 or more in a manner consistent with the response to an externally applied stress (Koenderink et al., 2009; Mizuno et al., 2008). The ability of force-generating elements to bring systems out of equilibrium has led to a lot of recent theoretical effort in predicting the phase behavior of actively driven matter using generalized statistical-mechanical frameworks (Marchetti et al., 2012). Moreover, theoretical studies of actin network mechanics have shown that these networks stiffen in response to internal, motor-induced stresses in a manner similar to the response to an external

stress (Sheinman et al., 2012a), unless these networks are poised near the rigidity percolation threshold (Sheinman et al., 2012b).

1.6 Thesis Outline

The goal of this thesis is to establish experimental systems which address major shortcomings in our current understanding of the properties of assemblies of biological polymers. A better grasp of the physical forces which organize biological polymers is necessary to account for the mutual interplay between cellular organization and force generation. In order to address these shortcomings, we perform experiments on model systems of reconstituted biological polymers which are 1. confined to cellular dimensions, 2. curved by crosslinks, and 3. driven by molecular motors. In all these cases, we lack a comprehensive theoretical understanding of the underlying physical mechanisms. The results we present should help advance our understanding of the role of the cytoskeleton in generating forces that underlie cellular organization.

In Chapters 2 and 3, we confine biopolymer systems to micrometer-sized spaces that mimic the spatial confinement that the cytoskeleton experiences within cells. Little is known about how polymers collectively organize when packed into a confined space with length scales similar to the contour length of the polymers themselves. In order to investigate the effect of an external geometry on polymer organization, we confine biopolymer systems in customized microchambers. In Chapter 2, we report experiments on nematic suspensions of the rod-like virus bacteriophage fd confined to shallow, donut-shaped microchambers. We quantify the nematic director field patterns and compare to predictions of Monte Carlo simulations accounting for the finite particle size. We find two patterns which are expected on the basis of continuum theory, but one pattern—with a striking three-fold-symmetry—is predicted to occur only for rods of finite length. In Chapter 3 we report experiments of actin networks confined to shallow, rectangular microchambers. We

quantify the nematic director field and find that nematic and bundled networks preferentially align along either the diagonal axis of the chambers, or parallel to chamber walls.

In Chapter 4, we investigate the actin-organizing capabilities of septins. A close interplay between septins and the actin cytoskeleton has been previously suggested, but little is known about the nature of this interaction. We report experiments which show that septins directly bind and bundle actin filaments, overthrowing prevailing assumptions that they do not interact directly. Surprisingly, we also find rings of actin bundles. Our observations demonstrate that septins alone are sufficient for actin ring formation, which may explain recent *in-vivo* experiments showing that septins are necessary for proper contractile ring formation in *Drosophila* embryos undergoing cellularization.

In Chapters 5 and 6, we investigate how myosin motors exert contractile forces on crosslinked actin filament networks. Little is known about the mechanisms whereby crosslinks transmit forces generated by molecular motors over macroscopic length scales. In Chapter 5, we show experimentally that myosin motors contract actin networks crosslinked by fascin to clusters with a scale-free size distribution. This critical behavior occurs over an unexpectedly broad range of crosslink concentrations. To understand this robustness, we compare our experimental results to a quantitative model of contractile networks. This model takes into account *network restructuring*: motors reduce connectivity by forcing crosslinks to unbind. Paradoxically, to coordinate global contractions, motor activity should be low. Otherwise, motors drive initially well-connected networks to a critical state where ruptures form across the entire network. In Chapter 6, we extend the results in Chapter 5 by comparing the effect of different crosslink proteins (fascin, fimbrin, and α-actinin). We furthermore vary motor activity by varying ATP and salt concentrations in the buffer, and vary network connectivity by varying actin filament density and length. We propose a phase-space diagram of connectivity-governed contractile active gels.